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Comparative effects of prolonged administration of cyanide, thiocyanate and chokecherry (*Prunus virginiana*) to goats†

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ABSTRACT: The aim of the present study was to determine and compare the clinical, hematological, biochemical and histopathological changes induced by cyanide, thiocyanate and chokecherry (*Prunus virginiana*) in goats. Sixteen Boer-Spanish cross-bred female goats were divided into four treatment groups: (1) control, (2) potassium cyanide (KCN) at 3.8 mg kg^{-1} day^{-1}, (3) potassium thiocyanate (KSCN) at 4.5 mg kg^{-1} day^{-1} and (4) ground frozen chokecherry leaves and flowers at a target dose of 2.5 mg HCN kg^{-1} day^{-1}, all for 4 weeks. Clinical signs were observed in two goats treated with chokecherry. Only sporadic changes were found in the hematological and blood chemical panel. Goats treated with chokecherry and thiocyanate had an increased number of vacuoles in the colloid of thyroid glands. Spongiosis and spheroids were found in the mesencephalon from goats treated with KCN and chokecherry. These findings suggest the thyroid lesions can be attributed to thiocyanate, whereas the effects on the nervous system were most likely caused by cyanide. Published in 2007 by John Wiley & Sons, Ltd.

KEY WORDS: cyanogenic plants; poisonous plants; cyanide; thiocyanate; *Prunus virginiana*; chokecherry; goats

Introduction

The production of cyanogenic glycosides in plant tissue is probably an important system of plant defense to minimize predation (Jones, 1998) and worldwide at least 2500 species of plants are known to be cyanogenic (Vetter, 2000). A common cyanogenic plant in North America is chokecherry (*Prunus virginiana*) that contains the cyanogenic glycoside prunasin (Majak, 1992). Cyanide inhibits several cellular enzymes including cytochrome oxidase, which is a key enzyme in the cellular respiratory chain (Ballantyne, 1987). Fortunately, mammals have robust systems to detoxify cyanide quickly; about 80% of absorbed cyanide is detoxified by enzymatic conversion into thiocyanate (Sousa *et al*., 2003). However, acute poisoning occurs when these systems are overwhelmed.

Chronic or long-term ingestion of cyanide has been linked to several neuronal, pancreatic and thyroidal disturbances (Kamalu, 1995; Jones, 1998). Though less toxic, thiocyanate has also been linked to hypothyroidism. Thiocyanate competes with iodide in the thyroid gland resulting in hypothyroidism (Delange and Ermans, 1996). Further, other deleterious effects may occur from cyanide toxicity. Little real evidence is available to explain the other neuronal and pancreatic effects of chronic poisoning. Some have speculated that the diabetogenic effect is due to direct injury of cyanide on the acinar portion of the pancreas, releasing digestive enzymes into the parenchyma that would affect pancreatic islets (McMillan and Geevarghese, 1979). The nervous system lesions have also been attributed to cyanide inhibition of oxidative phosphorylation and subsequent hypoxia/anoxia (Kamalu, 1995). Alternatively, it was suggested that thiocyanate increased glutamate affinity of AMPA receptors (Spencer, 1999). Thus, the extent to which thiocyanate contributes to chronic cyanide toxicity is not clear. Furthermore, it is not known whether cyanide or thiocyanate administration can reproduce all the chronic effects associated with chronic cyanide exposure.

The purpose of this work was to document the chronic effects of dosing cyanide and cyanogenic plants in goats and compare this toxicity with the lesions produced by thiocyanate intoxication. This was done by describing and comparing the clinical, hematological, biochemical and histopathological changes induced by potassium cyanide (KCN), potassium thiocyanate (KSCN) and chokecherry in goats. Our hypothesis was that thiocyanate administration would result in toxicity that closely mimics KCN and cyanogenic glycosides from fresh plant material.

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Materials and Methods

Plant Material

Small leaves and flowers of chokecherry (*Prunus virginiana*) were collected at Pineview Reservoir near Huntsville, Utah (N41″5.27′ W111″8.40′, elev. 1550 m), during the morning (1000 to 1100 h) on 30 April and 4 May 2001. Voucher specimens were deposited in the USDA-ARS herbarium. The plant material was placed in plastic bags, placed on dry ice in a cooler, and transported to the laboratory, where it was frozen at −20 °C until the start of the experiment.

The levels of HCN released by chokecherry were determined by the methodology proposed by Majak *et al*. (1980) and Lambert *et al*. (1975) using colorimetric measurement of cyanide with pyridine-barbituric acid for development of the color complex. Frozen chokecherry samples were ground and 100 mg of tissue was introduced into the main chamber of a 25 ml Erlenmeyer flask containing a center well with 0.4 ml 1 M NaOH. Three ml of phosphate buffer was added with 10 units of ß-glucosidase in the main compartment. The flask was immediately sealed and incubated at 35 °C for 16 h (overnight). The NaOH solution from the center well (0.4 ml) was then removed and transferred to 25 ml volumetric flasks, and 1 ml of succinimide reagent (5 g of N-chlorosuccinimide dissolved in 100 ml water, 0.5 g of succinimide added and diluted to 500 ml with deionized water) and 1 ml of barbituric acid-pyridine reagent (3 g of barbituric acid in around 15 ml of deionized water, 15 ml of pyridine and diluted to 50 ml with deionized water) was added, and further diluted to 25 ml with deionized water. The absorbance was measured on a spectrophotometer after 15 min at 575 nm. Prunasin (Sigma) was used as the standard. The prunasin concentration in the chokecherry samples averaged 3.2 g kg^{-1} with a range of 2.25–4.11 g kg^{-1}.

Animals and Experimental Design

Sixteen Boer-Spanish cross-bred female goats (10 months old; mean weight = 20 ± 3 kg) were divided into four equal groups, which were treated with: (1) ground hay and water only (control), (2) potassium cyanide (KCN) at 3.8 mg kg^{-1} day^{-1} (equivalent to 2.5 mg HCN kg^{-1} day^{-1}), (3) potassium thiocyanate (KSCN) at 4.5 mg kg^{-1} day^{-1} (equivalent to endogenous formation from 2.5 mg HCN kg^{-1} day^{-1}) and (4) ground frozen chokecherry (*Prunus virginiana*) leaves and flowers at a target dose of 2.5 mg HCN kg^{-1} day^{-1}. The administration of all substances was divided into two daily doses (0700 and 1500 h) given by oral gavage for 30 consecutive days. Doses corresponding to 2.5 mg HCN kg^{-1} day^{-1} and the length of treatment (30 days) were chosen according to earlier studies conducted by the authors. The chokecherry material was prepared by chopping the frozen plant material in a blender with the lowest amount of water possible (approximately 750 ml). The same volume of ground alfalfa hay mixed with water was given by gavage to controls. The goats were housed individually, with free access to water and food. They were fed a diet of commercial alfalfa pellets at 4% of body weight at 0800 each day; alfalfa pellets from this source typically have a concentration of 16% crude protein and 46% neutral detergent fiber (NDF) (Pfister, unpublished data). The food consumption was measured daily for each animal and dosed animals were closely monitored for poisoning. If clinical signs were severe (animals trembled excessively and became recumbent with mild convulsions) a single dose of an antidote for cyanide toxicity, sodium nitrite (10 g 100 ml^{-1} of distilled water, at 20 mg kg^{-1} body weight) and sodium thiosulfate (20 g 100 ml^{-1} of distilled water, at 500 mg kg^{-1} body weight) was given i.v.

Blood samples were collected on days 0, 3, 6, 9, 12, 15, 18, 21, 24, 27 and 30 via jugular venipuncture for determination of thiocyanate levels (plasma) and red and white blood cell counts (whole blood), and at 0, 15 and 30 days for a complete chemical panel (serum). The collection of blood was done before the animals were dosed or fed. Serum and plasma samples were maintained frozen at −20 °C until analyses. Whole blood samples were kept cold during collection and immediately submitted to measurement procedures. All procedures were approved by the Institutional Animal Care and Use Committee at Utah State University (protocol #1027).

Hemogram, Biochemical Panel and Thiocyanate Determination

Hematology analysis, which were performed with a impedance cell counter (Baker 9110, Biochem Immunosystems, Allentown, PA) included red blood cells (RBC) and white blood cells (WBC) counts, hematocrit (PCV), hemoglobin concentration (Hgb), red cell distribution width (RDW), and the hematimetric indices of mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC). Serum biochemistries were measured using a Beckman CX-5 automated chemistry analyser using Beckman reagents (Beckman Coulter, Fullertown CA). Determinations included alanine aminotransferase (ALT), aspartate aminotransferase (AST), γ-glutamyltransferase (GGT), creatine phosphokinase (CPK), alkaline phosphatase (ALP), lactate dehydrogenase (LD-L), total, direct and indirect bilirubin (TBIL, DBIL, IBIL), calcium (Ca), magnesium (Mg), potassium (K), sodium (Na), chloride (Cl), phosphorus (P), glucose, serum urea

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nitrogen (SUN), creatinine, albumin, total serum proteins (TP), albumin/globulin (A/G) ratio, bicarbonate (CO_2), osmolarity (Osm), anion gap and thyroxine (T4). Plasma thiocyanate concentrations were determined by spectrophotometry following the method of Pettigrew and Fell (1972) with minor modifications described earlier (Soto-Blanco and Górniak, 2003).

The range for normal values of hematology and biochemistry results were generated from controls and pretreatment samples with a 95% confidence interval.

Pathological Study

At the end of the experiment, all the goats were euthanized by i.v. injection of Terminol 3 (1 ml 4.5 kg^{-1}). Tissues from pancreas, thyroid gland, liver, kidney, lungs, heart, skeletal muscle, spleen, rumen, intestines, sciatic nerve and the whole central nervous system were collected for pathological study. Paraffin embedded sections were stained with hematoxylin and eosin (H & E). Nervous system tissues were stained with both H & E and luxol fast blue/periodic acid Schiff (LFB/PAS).

As there was a treatment related change in thyroid follicular epithelium resorption, morphometric evaluation was done by counting follicles with resorption vacuoles in the colloid and measuring the follicular size. Five hundred randomly selected follicles per animal were counted and the percentages of those with active resorption follicles were calculated. The follicular volumes were estimated by projecting thyroid micrographs onto a lattice and counting the chance intersections between the lines and the follicles. From these estimates, the ordinary mean volume (V_V), the volume-weighted mean volume (V_N), the standard deviation ($SD_N(v)$), and the variation squared ($CV_N^2(v)$) (Gundersen and Jensen, 1985; Baddeley *et al*., 1986; Sørensen, 1992) were calculated.

Statistical Analysis

The statistical design for the study was a completely randomized design with four treatments. Statistical analysis was done using a mixed linear model approach with SAS (SAS Statistical Software V8, 2000, SAS Institute Inc., Cary, NC) on all dependent variables. This analysis accounts for the increased probability of correlated measurements over time when taken from the same animal. Animals were considered as a random factor, with each animal nested within treatments, with repeated measurements over time. Various mixed models (e.g. compound symmetry, unstructured, autoregressive) were compared to determine the covariance structure, and the best fitting model was determined for each dependent variable. Significant day × treatment interactions were examined using the PDIFF procedure of SAS with preplanned comparisons. Normal ranges for hematology and biochemistry results were generated from controls and pretreatment samples with a 95% confidence interval.

Results

There was no difference ($P > 0.05$) between treatment groups in weight gain, food intake and food conversion (Table 1). Most animals were clinically normal; however, one goat developed moderate trembling and vocalizations after 4 and 5 days of chokecherry dosing. On day 6 this goat developed convulsions and was given a single dose of the antidote. On day 7 and in all subsequent dosing this goat was given 75% of the chokecherry dose. She developed no more clinical signs and additional convulsions were not detected. Another goat from the same group developed mild superficial trembling on days 6–9. She apparently adapted to the chokecherry dose and no clinical signs were noted thereafter.

Table 1. Body weight gain, food consumption, food conversion, thyroid weight and follicular size of goats treated with KCN, KSCN or chokecherry for 30 days. Controls were dosed with alfalfa hay. Data are presented as mean ± SEM; $n = 4$

	Control	KCN	KSCN	Chokecherry
Weight gain (kg)	5.42 ± 0.60	4.47 ± 0.18	2.52 ± 1.05	3.15 ± 2.52
Weight gain/initial weight (kg 100 kg^{-1})	23.3 ± 3.29	19.5 ± 0.89	10.7 ± 4.14	13.8 ± 4.5
Food consumption (kg)	31.0 ± 1.96	30.3 ± 0.56	23.3 ± 3.88	26.2 ± 3.16
Daily food consumption (g)	1033 ± 65.5	1011 ± 18.6	777 ± 129.3	874 ± 105.2
Daily food consumption/body weight (g kg^{-1})	40.4 ± 1.12	40.4 ± 1.81	31.5 ± 3.61	34.4 ± 2.70
Food conversion (kg kg^{-1})	5.88 ± 0.61	6.81 ± 0.31	*8.19 ± 1.25	11.5 ± 4.60
Total thyroid weight (g)	2.18 ± 0.14	2.37 ± 0.29	2.78 ± 0.47	2.52 ± 0.26
Thyroid weight/body weight (g kg^{-1})	7.48 ± 0.25	8.58 ± 0.86	10.88 ± 2.06	9.29 ± 1.07
Follicles with vacuoles	[a]20.4 ± 7.51	[b]17.3 ± 5.20	32.9 ± 5.81	[a,b]57.2 ± 7.05
V_V	32.6 ± 3.00	31.5 ± 0.54	38.2 ± 5.15	30.0 ± 1.73
V_N	43.2 ± 6.77	47.9 ± 1.37	57.8 ± 8.03	46.4 ± 1.89
$SD_N(v)$	22.4 ± 2.32	22.7 ± 0.97	27.3 ± 3.93	22.1 ± 0.78
$CV_N^2(v)$	10.1 ± 0.79	9.05 ± 0.87	8.92 ± 0.80	9.40 ± 0.57

* $n = 3$ for this variable within this treatment group (one goat did not gain any weight throughout experiment).
[a,b] Means that are not preceded by common superscript letters are significantly different ($P < 0.05$).

Table 2. Plasma levels of thiocyanate (mmol l^{-1}) in goats treated with KCN, KSCN or chokecherry for 30 days. Controls were dosed with alfalfa hay. Data are presented as mean ± SEM; $n = 4$

Days of dosing	Control	KCN	KSCN	Chokecherry
0	36.4 ± 3.06	31.0 ± 4.04	29.8 ± 1.83	38.4 ± 2.95
3	[a]36.7 ± 2.03	[b]119.8 ± 26.3	[b]132.4 ± 20.3	[c]207.0 ± 57.3
6	[a]39.6 ± 2.21	[b]104.7 ± 8.86	[c]152.9 ± 10.0	[d]215.7 ± 25.7
9	[a]36.5 ± 3.89	[a]81.7 ± 20.4	[b]133.3 ± 22.0	[b]170.2 ± 14.5
12	[a]35.9 ± 1.38	[b]105.8 ± 25.9	[c]137.9 ± 27.2	[c]170.3 ± 16.9
15	[a]33.5 ± 1.97	[b]109.2 ± 14.0	[b]136.8 ± 25.3	[b]154.8 ± 11.8
18	[a]37.5 ± 1.98	[a,b]79.3 ± 10.3	[b,c]124.0 ± 12.6	[c]168.6 ± 12.9
21	[a]36.3 ± 2.41	[b]97.3 ± 11.5	[b]131.0 ± 16.7	[c]199.8 ± 18.0
24	[a]39.0 ± 3.88	[b]124.7 ± 21.3	[b,c]155.6 ± 25.7	[c]194.2 ± 20.9
27	[a]40.8 ± 3.35	[a]56.7 ± 8.82	[b]158.8 ± 12.8	[b]170.9 ± 11.5
30	[a]36.5 ± 3.04	[a]53.1 ± 7.11	[b]106.7 ± 13.9	[c]186.8 ± 19.4
Range of normal values	34.7–37.8 mmol l^{-1}			

[a,b,c,d] Means in the same row preceded by different superscript letters are significantly different ($P < 0.05$).

Plasma thiocyanate concentrations were significantly different for all treatments (KCN, KSCN and chokecherry) (Table 2). The thiocyanate and chokecherry groups generally had higher concentrations of thiocyanate than did the controls and KCN treatment groups throughout much of the dosing period. The plasma thiocyanate concentration in goats dosed with cyanide varied over time, and generally exceeded concentrations found in controls, except for days 27 and 30. Interestingly, the goat from the chokecherry group that developed convulsions and subsequently received a 75% dose had plasma thiocyanate concentrations higher than the mean value of the other animals in the same group (Fig. 1).

The hematological analyses showed only occasional differences in RBC, Hgb, PCV and RDW (Table 3); no significant difference was found in MCV, MCH, MCHC and WBC. Serum concentrations of Cl were increased ($P < 0.05$) in all cyanide-treated groups compared with the controls on day 30. Only occasional differences were found in Ca, K, Na, TBIL, glucose, TP, albumin, SUN, creatinine, CO_2 and Osm levels, CPK activities, and albumin/globulins and urea nitrogen/creatinine ratios (Tables 4 and 5). These changes were sporadic and did not indicate specificity in organ damage or dysfunction. No significant difference between treatments was found in ALT, AST, GGT, ALP, LD-L, DBIL, IBIL, Mg, P, A/P ratio, anion gap or T4.

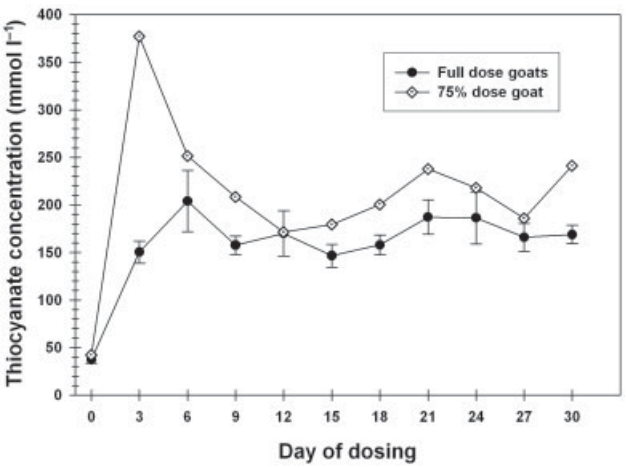

Figure 1. Plasma levels of thiocyanate (mmol l^{-1}) in goats treated for 30 days with chokecherry at a target dose of 2.5 mg HCN kg^{-1} day^{-1} (3 goats) or 75% of the target dose (one goat). The 75% dose goat had an adverse reaction to the full dose, and after day 7 was given a partial dose

At necropsy, no macroscopic lesions were found in any tissue, except for pale thyroid glands from a chokecherry-treated goat. The weights of the thyroids were not different ($P > 0.05$) in all groups (Table 1). Histological changes were found only in thyroid and nervous tissues. The lesions in thyroid glands consisted of an increased number of vacuoles in the colloid of goats treated with KCN, KSCN and chokecherry. However, only the chokecherry group showed an increase ($P < 0.05$) in the percentage of follicles containing vacuoles compared with the control group. On the other hand, no significant difference was found in V_V, V_N, $SD_N(v)$ and $CV_N^2(v)$ (Table 1). Nervous system tissue showed evidence of spongiosis and spheroids in the mesencephalon from goats treated with KCN and chokecherry; these findings were more severe in chokecherry than in KCN-treated goats.

Discussion

In rats pure prunasin is much less toxic than equivalent cyanide doses as 30% to 45% is excreted intact (Sakata *et al*., 1987). However, this toxicity is regained when cyanide is released by hydrolysis that is facilitated by plant enzymes. Many of these enzymes are activated when plants are stressed or damaged. Rumen bacteria

Table 3. Hematological changes in goats treated with KCN, KSCN or chokecherry for 30 days. Controls were dosed with alfalfa hay. Data are presented as mean ± SEM; $n = 4$

	Day 0	Day 6	Day 9	Day 27	Day 30	Range for normal values
RBC ($\times 10^6$ mm^{-3})						
Control	15.8 ± 0.42	[a]12.4 ± 0.66	[a]11.7 ± 0.39	12.1 ± 0.85	14.7 ± 0.77	13.1–14.4
KCN	16.3 ± 1.82	13.6 ± 1.22	12.9 ± 1.14	12.7 ± 0.95	16.2 ± 1.64	$\times 10^6$ mm^{-3}
KSCN	17.5 ± 0.85	[b]15.6 ± 0.88	[b]14.3 ± 0.59	14.2 ± 1.22	16.2 ± 1.16	
Chokecherry	17.5 ± 0.90	13.7 ± 0.64	13.3 ± 0.84	12.3 ± 0.55	15.5 ± 1.03	
Hgb (g%)						
Control	11.2 ± 0.34	9.6 ± 0.31	8.9 ± 0.27	9.1 ± 0.26	10.6 ± 0.20	9.7–10.3
KCN	11.2 ± 0.49	9.8 ± 0.31	9.2 ± 0.41	9.3 ± 0.27	11.1 ± 0.60	g%
KSCN	11.4 ± 0.26	10.4 ± 0.57	9.9 ± 0.47	[a]9.9 ± 0.35	11.0 ± 0.32	
Chokecherry	11.2 ± 0.41	9.6 ± 0.15	9.3 ± 0.23	[b]8.9 ± 0.16	10.7 ± 0.34	
PCV (%)						
Control	27.3 ± 0.87	[a]21.4 ± 1.26	[a]20.1 ± 0.81	21.4 ± 1.67	26.2 ± 1.61	22.8–25.2
KCN	28.3 ± 3.39	23.4 ± 2.31	22.2 ± 2.10	22.7 ± 2.02	29.1 ± 3.28	%
KSCN	31.0 ± 1.67	[b]27.4 ± 1.50	[b]25.1 ± 0.96	25.1 ± 2.63	28.7 ± 2.46	
Chokecherry	30.7 ± 1.61	24.1 ± 1.23	23.3 ± 1.62	22.1 ± 1.16	28.2 ± 2.21	
RDW (%)						
Control	43.8 ± 0.39	42.6 ± 0.40	42.2 ± 0.27	43.2 ± 0.45	44.7 ± 0.31	43.1–43.6
KCN	43.3 ± 0.33	42.1 ± 0.40	41.7 ± 0.38	43.5 ± 0.87	44.8 ± 0.69	
KSCN	44.0 ± 0.17	42.5 ± 0.95	42.3 ± 0.95	42.5 ± 0.52	[a]43.4 ± 0.41	
Chokecherry	43.4 ± 0.79	42.8 ± 0.20	42.8 ± 0.30	44.0 ± 0.83	[b]45.4 ± 0.96	

[a] Means for each variable and in the same column preceded by different superscript letters are significantly different ($P < 0.05$).

also promote hydrolysis of cyanogenic glycosides (Majak and Cheng, 1984). Individual variation in toxicity may be due to differences in these hydrolytic enzymes. In our study, one goat treated with chokecherry seemed much more sensitive. Others have speculated, for cattle, that such animals have a more efficient mechanism for releasing cyanide from the plant material (Majak *et al*., 1980; Majak, 1992). Such animals are probably those that are likely to become poisoned clinically. Additional work may be indicated to better determine why some animals are more susceptible and to determine if these animals can be identified or otherwise treated to better protect them from poisoning.

The clinical signs of toxicity were seen in these sensitive goats 4 and 5 days after dosing was initiated. Similar delays have been observed in goats receiving KCN (Soto-Blanco *et al*., 2001a; 2005; Soto-Blanco and Górniak, 2004) and in sheep dosed with the cyanogenic glycoside amygdalin (Villalba *et al*., 2002). One explanation for these delayed signs might be a transitory exhaustion of the cyanide detoxification mechanisms. The plasma thiocyanate concentrations seen in the group treated with KCN may support this hypothesis as the concentrations abruptly decreased after the initial increases in plasma thiocyanate concentrations through the first days of the study. Alternatively the delayed onset of clinical signs could be related to toxin distribution. At these doses it may take several days of accumulation for cyanide to reach a toxic concentration in neurons. In fact, neuronal accumulation of labeled cyanide has been documented *in vitro* (Borowitz *et al*., 1994), but little is known of cyanide toxicokinetics on a cellular basis.

Several studies have shown that chronic cyanide exposure resulted in reduced body weight and lower weight gains in several species including broilers (Panigrahi *et al*., 1992), rats (Sousa *et al*., 2002), dogs (Ibebunjo *et al*., 1992), pigs (Tewe *et al*., 1984), sheep (Onwuka *et al*., 1992) and goats (Soto-Blanco *et al*., 2001a). On the contrary, animal weight gains, food consumption, feed conversion and efficiency were not affected by cyanide, thiocyanate or chokecherry treatment in this study. Three hypotheses have been proposed to explain the negative effects of cyanide on weight gains: (1) depletion of the sulphur-containing amino acids caused by their use as a source of sulphur for cyanide detoxification, (2) impaired secretion of growth hormone and reduced number of growth hormone receptors secondary to hypothyroidism (Soto-Blanco *et al*., 2001a) and (3) impaired cellular energy metabolism mediated by cyanide inhibition of mitochondrial oxidative phosphorylation (Sousa *et al*., 2002). Numerically, it was found that body weight gain and food consumption were lower and food conversion was higher in cyanide-treated goats than in controls. The limited sample size, time of exposure and inherent variability in feed intake probably precluded finding treatment differences.

Long-term cyanogenic plant consumption by both humans (Adewusi and Akindahunsi, 1994) and animals (Bahri, 1987; Kamalu and Agharanya, 1991) has been linked to the development of hypothyroidism and goiter. In the present work, goats treated with chokecherry and thiocyanate had an increased number of vacuoles in the colloid of thyroid glands, which was similar to earlier studies (Soto-Blanco *et al*., 2001a; Soto-Blanco and

Table 4. Serum biochemical changes in goats treated with KCN, KSCN or chokecherry for 30 days. Controls were dosed with alfalfa hay. Data are presented as mean ± SEM; $n = 4$

Treatment	Ca (mg dl^{-1})	K (mg dl^{-1})	Na (meq l^{-1})	Cl (meq l^{-1})	TBIL (mg dl^{-1})	Glucose (mg dl^{-1})	CPK (U l^{-1})
Day 0							
Control	[a]10.2 ± 0.28	[a,b]5.67 ± 0.09	147.4 ± 1.44	111.0 ± 0.47	0.40 ± 0.04	64.0 ± 2.12	65.8 ± 9.36
KCN	[b]9.25 ± 0.42	[a]5.18 ± 0.20	148.0 ± 1.60	111.7 ± 1.15	0.43 ± 0.02	57.8 ± 4.59	80.3 ± 9.36
KSCN	[a,b,c]10.1 ± 0.29	[b]6.29 ± 0.46	147.7 ± 2.35	114.0 ± 1.36	0.50 ± 0.04	70.8 ± 4.52	73.3 ± 5.72
Chokecherry	[a,c]10.5 ± 0.31	[a,b]5.94 ± 0.35	147.7 ± 1.03	111.3 ± 1.63	0.48 ± 0.03	61.0 ± 6.63	62.8 ± 3.64
Day 18							
Control	10.7 ± 0.17	5.09 ± 0.08	146.3 ± 0.75	113.9 ± 0.72	0.28 ± 0.02	73.0 ± 2.12	70.3 ± 11.0
KCN	[a]10.1 ± 0.25	5.16 ± 0.20	146.6 ± 1.04	114.0 ± 0.94	0.33 ± 0.05	60.0 ± 2.45	72.5 ± 10.9
KSCN	10.4 ± 0.16	5.65 ± 0.24	147.0 ± 1.43	114.1 ± 0.45	0.38 ± 0.06	74.0 ± 1.47	65.3 ± 13.7
Chokecherry	[b]11.0 ± 0.38	5.47 ± 0.18	146.5 ± 0.94	113.1 ± 0.43	0.33 ± 0.05	75.0 ± 1.87	56.3 ± 4.87
Day 30							
Control	11.3 ± 0.25	6.43 ± 0.44	[a]151.8 ± 1.39	[a]114.1 ± 1.24	[a]0.35 ± 0.05	[a]79.0 ± 1.08	[a]139.5 ± 20.1
KCN	11.1 ± 0.24	6.57 ± 0.39	[a,b]154.4 ± 1.00	[b]117.7 ± 1.65	[a]0.35 ± 0.03	[a,b]98.8 ± 10.1	[a,b]173.0 ± 19.7
KSCN	11.4 ± 0.51	6.89 ± 0.48	[b]156.1 ± 1.28	[c]124.3 ± 0.98	[b]0.48 ± 0.05	[b]114.3 ± 10.0	[b]189.3 ± 44.4
Chokecherry	12.0 ± 0.24	6.28 ± 0.42	[b]156.1 ± 1.62	[b]118.1 ± 1.04	[b]0.48 ± 0.03	[a,b]96.3 ± 20.9	[a]124.5 ± 9.42
Normal ranges							
Range for normal values	9.99–10.69 mg dl^{-1}	5.44–6.08 mg dl^{-1}	146.8–149.5 meq l^{-1}	111.6–113.7 meq l^{-1}	0.36–0.45 mg dl^{-1}	63.2–71.9 mg dl^{-1}	67.9–95.9 U l^{-1}

[a,b,c,d,e] Means for each day in the same column preceded by different superscript letters are significantly different ($P < 0.05$).

Table 5. Serum biochemical changes in goats treated with KCN, KSCN or chokecherry for 30 days. Controls were dosed with alfalfa hay. Data are presented as mean ± SEM; $n = 4$

Treatment	TP (g dl^{-1})	Albumin (g dl^{-1})	A/G Ratio	SUN (mg dl^{-1})	Creatinine (mg dl^{-1})	Bun/Crea	CO_2 (meq l^{-1})	Osm (mOs kg^{-1})
Day 0								
Control	[a]6.48 ± 0.18	1.53 ± 0.09	0.31 ± 0.01	23.8 ± 1.31	[a]0.7 ± 0.0	[a,b]28.5 ± 2.35	25.0 ± 0.26	294.1 ± 2.71
KCN	[a]5.98 ± 0.23	1.43 ± 0.11	0.31 ± 0.02	19.5 ± 0.29	[a,b]0.75 ± 0.03	[a,b]26.6 ± 1.71	23.8 ± 0.70	284.4 ± 3.28
KSCN	[b]6.88 ± 0.18	1.50 ± 0.08	0.28 ± 0.02	18.3 ± 1.49	[b,c]0.9 ± 0.04	[c]20.8 ± 0.82	23.0 ± 0.23	294.0 ± 4.92
Chokecherry	[ab]6.35 ± 0.23	1.45 ± 0.03	0.30 ± 0.02	19.0 ± 1.47	[a,b]0.75 ± 0.05	[b,c]25.2 ± 1.29	24.8 ± 1.60	293.9 ± 1.78
Day 18								
Control	[a,b]6.63 ± 0.17	[a,b]1.83 ± 0.06	[a,b]0.33 ± 0.01	[a]26.0 ± 0.62	[a,b]0.83 ± 0.08	33.9 ± 3.09	26.1 ± 1.08	294.5 ± 1.19
KCN	[a]6.35 ± 0.18	[a,b]1.68 ± 0.03	[a]0.36 ± 0.01	[a]28.3 ± 1.41	[a,b]0.83 ± 0.06	34.5 ± 3.57	25.0 ± 0.44	285.4 ± 2.13
KSCN	[a,b]7.00 ± 0.29	[a]1.55 ± 0.06	[b]0.29 ± 0.01	[b]35.5 ± 2.75	[c]1.03 ± 0.05	34.8 ± 2.81	26.5 ± 0.47	299.3 ± 2.19
Chokecherry	[b]7.13 ± 0.20	[b]1.80 ± 0.04	[a,b]0.33 ± 0.01	[a]27.5 ± 1.71	[a,b,c]0.9 ± 0.07	31.2 ± 0.79	25.3 ± 0.53	285.4 ± 2.32
Day 30								
Control	7.50 ± 0.18	1.98 ± 0.11	[a]0.36 ± 0.02	31 ± 2.38	[a]1.03 ± 0.06	[a,c]30.5 ± 0.78	[a]22.2 ± 1.06	[a]306.6 ± 3.34
KCN	7.60 ± 0.20	2.00 ± 0.04	[a]0.36 ± 0.01	30.5 ± 1.49	[a]1.1 ± 0.04	[a,b]28.0 ± 1.11	[a]21.1 ± 1.43	[a,c]312.7 ± 2.16
KSCN	8.00 ± 0.17	1.85 ± 0.06	[b]0.31 ± 0.01	33.8 ± 4.52	[b]1.35 ± 0.10	[b]24.7 ± 1.75	[c]15.9 ± 1.07	[b]321.4 ± 3.93
Chokecherry	8.15 ± 0.41	2.13 ± 0.10	[a]0.36 ± 0.01	34.5 ± 1.85	[a]1.13 ± 0.07	[a,c]31.0 ± 1.01	[a]20.3 ± 1.05	[b,c]317.1 ± 3.22
Normal ranges								
Range for normal values	6.38–6.88 (g dl^{-1})	1.48–1.69 (g dl^{-1})	0.30–0.33	20.2–24.7 (mg dl^{-1})	0.76–0.89 (mg dl^{-1})	25.4–29.8	23.2–25.0 (meq l^{-1})	293.2–299.4 (mOs kg^{-1})

[a,b,c] Means for the same day preceded by different superscript letters are significantly different ($P < 0.05$).

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Gorniak, 2003). There were no changes in thyroid weights or estimates of thyroid function (T4). These lesions suggest that this may be an initial thyroid perturbation, and if treatment was prolonged, thyroid dysfunction may occur. The results suggest that the evaluation of the extent of vacuolation of the thyroid is an initial step in determining the effects on thyroid metabolism. Even so, the increase in thyroid vacuoles in thiocyanate-treated goats points to thiocyanate being responsible for the action on thyroid tissue. In fact, thiocyanate is thought to compete with iodide on its capture from the bloodstream by thyroidal cells, resulting in reduced iodide in these cells and decreased thyroid hormone synthesis (Delange and Ermans, 1996). Furthermore, almost no thiocyanate is converted back to cyanide in the organism (Ballantyne, 1987). Additional studies using species-specific assays of thyroid function are needed to determine the involvement of these morphological changes in thyroid disease and the duration of poisoning required to develop thyroid dysfunction.

It has been purposed that chronic cyanide exposure can promote pancreatic lesions and diabetes (McMillan and Geevarghese, 1979; Petersen, 2002). However, no toxic effect was found in the pancreas, agreeing with earlier studies (Soto-Blanco *et al*., 2001b, 2002a). Furthermore, some authors have reported damage in muscle, heart, liver, kidneys, lungs and adrenal glands (Kamalu, 1993), but no lesions were found in these tissues in this study.

Hematological changes were linked to cyanide exposure in humans (El Ghawabi *et al*., 1975) and goats (Soto-Blanco *et al*., 2001a). The development of normocytic normochromic anemia in goats receiving KCN for 5 months was attributed to hypothyroidism or a direct interference of cyanide on erythropoiesis (Soto-Blanco *et al*., 2001a). The normal hematological results in this study exclude the possibility of a direct action of cyanide on erythropoiesis. Furthermore, the absence of anemia would be a consequence of non-disturbance of thyroidal metabolism.

Some authors have noted changes in serum biochemical panels in cyanide-treated animals (Kamalu, 1993; Okolie and Osagie, 1999). Kamalu (1993) noted that dogs had a reduction in serum levels of calcium and potassium, and variable effects on total proteins, albumin and globulins (Kamalu, 1993). In rabbits, serum activities of lactate dehydrogenase, sorbitol dehydrogenase, alanine aminotransferase and alkaline phosphatase and serum levels of urea and creatinine were affected by cyanide (Okolie and Osagie, 1999). In our study, the serum biochemical panel presented no definitive results except for sporadic changes in some parameters.

Long-term cyanide exposure is responsible for degenerative changes in the central nervous system in both humans (Osuntokun, 1981; Tylleskar *et al*., 1995) and animals (Soto-Blanco *et al*., 2002b). Nervous system tissue had spongiosis and spheroids in the mesencephalon from goats treated with KCN and chokecherry. In an earlier experiment, goats treated chronically with KCN developed axonal dystrophy (spheroids) and spongiosis and gliosis in the medulla oblongata, gliosis in pons and Purkinje cell degeneration in the cerebellum (Soto-Blanco *et al*., 2002b). The differences in the pathological findings are due to differences in gender, dose and treatment length. The earlier study used growing male goats that received lower doses of KCN (up to 3.0 mg kg^{-1} day^{-1}) for 5 months.

As neurons have relatively high metabolic rates with little capacity for anaerobic metabolism, cyanide may promote neurotoxic effects through inhibition of cellular respiration. Furthermore, cyanide interferes with several neurotransmitters including γ-aminobutyric acid — GABA (Tursky and Sajter, 1962; Cassel *et al*., 1991), glutamic acid (Cassel *et al*., 1991), acetylcholine (Owasoya and Iramain, 1980), dopamine (Cassel *et al*., 1995), excitatory amino acids (McCaslin and Yu, 1992; Gunasekar *et al*., 1996) and nitric oxide (Gunasekar *et al*., 1996). Even though thiocyanate increases the affinity of AMPA receptors for glutamate (Spencer, 1999), it probably does not contribute to cyanide neurotoxicity because thiocyanate-treated goats had no lesions in nervous system tissues. In the same way, the cyanogenic glycoside prunasin probably does not have direct effects on nervous system tissues because there were no additional lesions beyond those promoted by cyanide in goats treated with chokecherry.

In conclusion, these findings suggest that the cyanogenic glycoside prunasin is probably not directly toxic, and chokecherry-related toxicity under these conditions can be attributed to cyanide released from glycosides or to thiocyanate formed from cyanide. They also suggest the effects on thyroid glands were produced by thiocyanate. In contrast, the nervous system lesions were probably the direct effect of cyanide. Additional work is needed to determine if the thyroid lesions are likely to cause thyroid dysfunction and the impact of chronic intoxication with cyanogenic glycosides on animal growth and production.

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